Development on a commercial product of plant immunity intensifying *Bacillus amyloliquefaciens*

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1 Introduction

In recent years, application of antagonistic microbes exhibits its potential for reducing the usage of fungicide. It have been proven that various plant diseases are controlled by microbes such as *Trichoderma* spp., *Pseudomonas* spp., *Streptomyces* spp., and *Bacillus* spp. Among them, *Bacillus* spp. is most common due to it can produce endospore to increase the shelf-life of commercial products. Most of the cases show the possible mechanisms of *Bacillus* spp. on control plant diseases are associated with the anti-microbial compounds produced by these bacteria, such as *B. amyloliquefaciens* strain PMB04. Therefore, inhibitory effect of *Bacillus* spp. against plant pathogens carried out on agar plate is used to screen potential strains. However, other methods to select bacterial strains on disease control in the filed still need to be developed. In our laboratory, we already established a novel method to screen bacterial strains which can intensify plant immune responses. The *Bacillus* strains, *B. amyloliquefaciens* PMB05, screened from this method control plant diseases effectively. For example, the fruit blotch on watermelon, anthracnose of strawberry, and bacterial canker on lemon controlled by PMB05 has been demonstrated. Here we show *B. amyloliquefaciens* strains, PMB04 and PMB05, are powerful strains on control plant diseases. And, these two strains are worthwhile for commercial production.

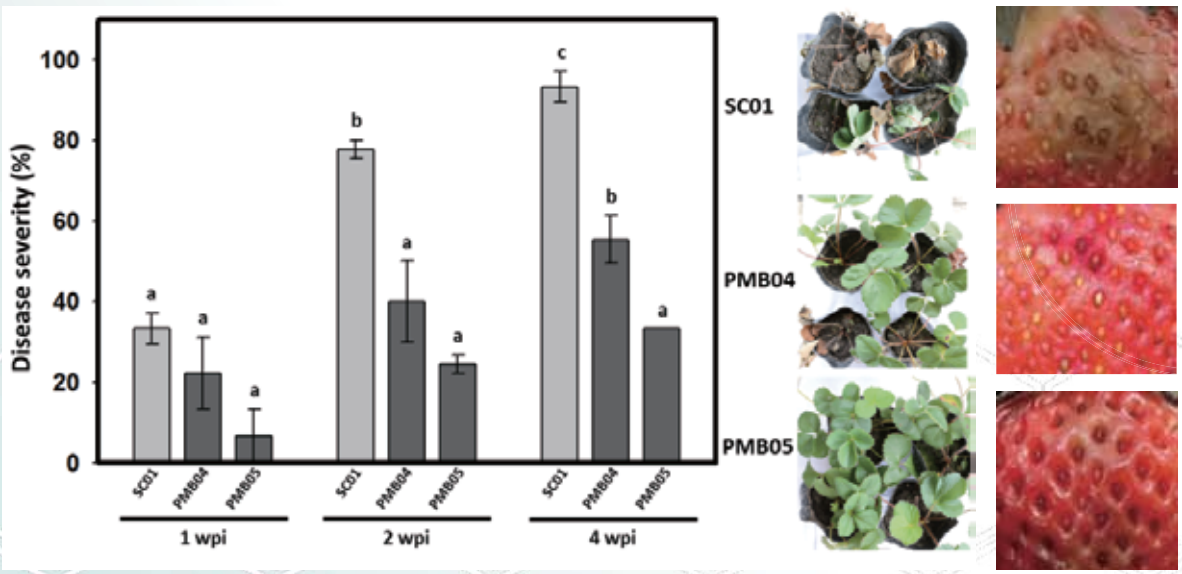


Figure 1 Disease control on anthracnose of strawberry carried out with *Bacillus amyloliquefaciens* strains.

2 Design Concept

As previous description, *Bacillus amyloliquefaciens* strain PMB04 produces anti-microbial compounds to reduce the growth of pathogen and further control plant diseases. The other strain, PMB05 intensifies plant immune responses to enhance disease resistance against plant pathogens. These two cases are worthy to develop commercial products.

3 Technical Development

The basic fermentation formula of *Bacillus amyloliquefaciens* PNB05 has been developed.

Figure 2 Disease control on bacterial fruit blotch of watermelon carried out with *Bacillus amyloliquefaciens* strains.

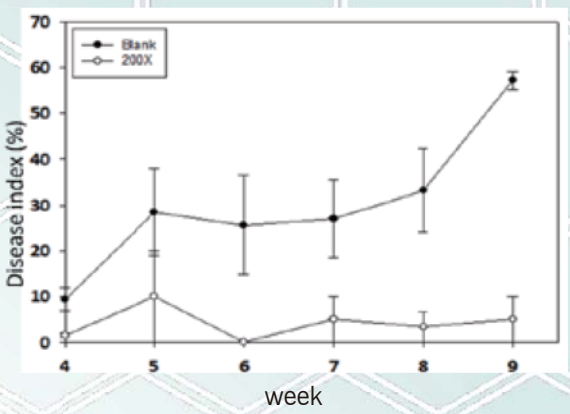


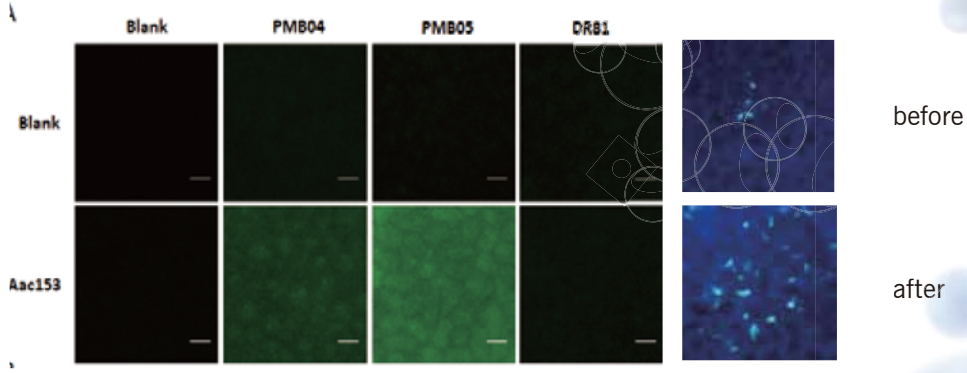
Figure 3 Disease control on bacterial canker of lemon in the filed with frementation liquid of *Bacillus amyloliquefaciens* strain PMB05.

4 Technological Competitiveness

Usage of chemical pesticides to control plant disease is highly depended in agricultural system. However, inappropriate application brings out the concerns on environment and food safety. To our knowledge, *Bacillus amyloliquefaciens* is considered as a bacterium with safety to human. Therefore, application of *Bacillus amyloliquefaciens* strains in agricultural system is a possible strategy.

5 R&D Result

In our study, we use *Bacillus amyloliquefaciens* strains, PMB04 and PMB05, to control anthracnose of strawberry and bacterial fruit blotch on watermelon. Anthracnose is a devasting fungal disease on strawberry production caused by *Colletotrichum gloeoesporioides*. The *in vitro* inhibitory assay revealed that the hyphal growth of *C. gloeosporioide* SC01 was inhibited by *B. amyloliquefaciens* PMB04 and PMB05. We further found that the cultural filtrate from PMB04 was lethal to the conidia of SC01. These results suggested that PMB04 and PMB05 would be applied to control anthracnose on strawberry. Biocontrol efficacy on strawberry fruits exhibited the symptoms was significant suppressed by these three strains. Moreover, the disease severity on strawberry seedlings was strongly reduced by PMB04 and PMB05. To gain more insight of these antagonistic on plant immune system, the intensification of PAMP-triggered immunity was assayed through observing rapid ROS generation and callose deposition. Result showed the immunity was only intensified by the cells of PMB05 upon the inoculation of SC01.



We concluded that lethal on conidia and intensification on plant immune responses were two important mechanisms to control strawberry anthracnose caused by *Colletotrichum gloeoesporioides*. In the other experiment, we shifted to focus on bacterial fruit blotch (BFB) caused by *Acidovorax citrulli*. This disease is an important limiting factor on watermelon production. Since the infested seeds can be the primary inoculum to cause epidemics in field, reducing the development of pathogen on seedlings becomes a possible strategy to control BFB. As describe above, two antagonistic strains, PMB04 and PMB05 were selected to apply on *A. citrulli*-infested seeds. Results revealed that the disease incidence and disease severity were significant reduced. Among them, weak antagonistic PMB05 exhibited strong control efficacy against BFB of watermelon rather than strong antagonistic strain PMB04. To realize the interaction between antagonistic strains and innate immunity of watermelon, signals of plant immunity intensified by antagonistic strains were assayed. Results revealed that both rapid ROS generation and callose deposition were significant intensified by PMB05 upon the inoculation of *A. citrulli*. Therefore, we concluded that antagonistic effect and priming plant immunity of *B. amyloliquefaciens* strains bring the potential of developing commercial biocontrol products.

6 Acknowledges

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7 References

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